

Genetic Regulation of Endotoxin-Induced Airway Inflammation

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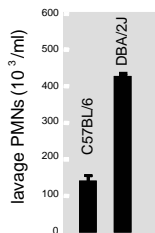
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Background: Lipopolysaccharide (LPS)-induced inflammation is an important aspect of diseases as diverse as asthma and sepsis. An improved understanding of biologic responses to LPS might lead to better therapies for these and other diseases.

Methods: A genomic scan was first used to identify quantitative trait loci (QTLs) regulating the biologic response to LPS. C57BL/6J x DBA/2J(BXD) F2 mice were exposed to an aerosol of LPS and their lungs lavaged to determine levels of inflammatory cells and cytokines in the airway. Separate pools of DNA were prepared from high- and low-responder mice, respectively, and these pools were analyzed for the relative abundance of each strain's DNA at 300 loci throughout the genome. Significant divergence from the expected 50% for each strain was diagnostic of a QTL. The QTLs emerging from this analysis were further narrowed using the recombinant inbred strain test (RIST). Microarray-based expression analyses were used to identify LPS-regulated genes.

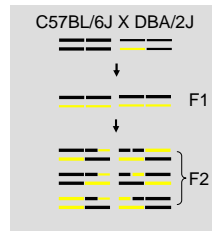
Results: A QTL affecting pulmonary tumor necrosis factor (TNF)-alpha production was identified on chromosome 2, and a separate QTL affecting both polymorphonuclear leukocyte recruitment and TNF-alpha levels was identified on chromosome 11. Microarray analyses of unchallenged and LPS-challenged mice identified genes whose LPS-induced gene expression differed between the strains under study. Genes having differential expression and that are located within the identified QTLs are high-priority candidates for functional studies. Additional high-priority candidates were identified based on DNA polymorphisms of genes lying within these QTLs.

- 1) C57BL/6 and DBA/2J mice have quantitatively different responses to inhaled endotoxin



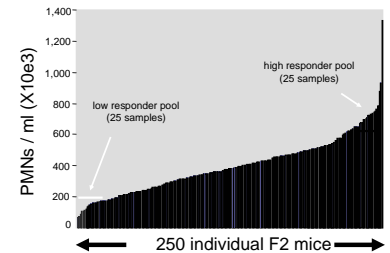
This difference shows that genetic factors cause differential responsiveness to inhaled endotoxin

- 2) Generate F2 [C57BL/6 X DBA/2J] mice



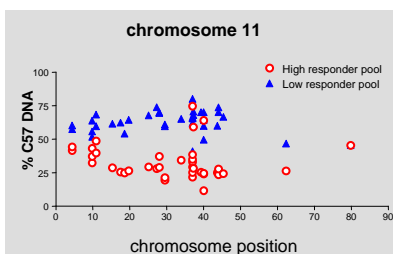
Note that although all F1 mice are genetically identical, all F2 mice are genetically different

- 2) Analyze response of F2 [C57BL/6 X DBA/2J] mice to inhaled endotoxin



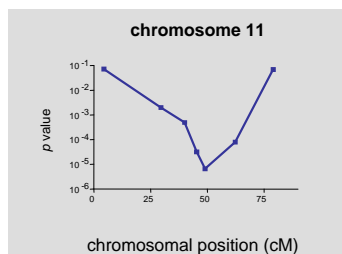
This variable responsiveness suggests that identification of parental strain DNA in low and high responder mice can be used to identify chromosomal loci affecting this quantitative trait (QTL).

- 3) Comparison of parental strain DNA contribution in pools of DNA from high and low responder mice



Note that the low responder pool contains more C57 DNA at this locus than the high responder pool

- 4) Correlation of genotype to phenotype using individual mice and a select group of genetic markers



A single locus near cM 50 is highly correlated with endotoxin-induced PMN infiltration to the lung

- 5) Genes within QTL that have polymorphisms between C57BL/6 and DBA/2J

Gene name	Annotation
Zfp39	zinc finger protein 39
Myblp1a	MYB binding protein (P160) 1a
Pkd2	phospholipase D2
Abr NM-198018	active BCR-related gene
Ap2b1	adaptor-related protein complex 2, beta 1 subunit
Expi	extracellular proteinase inhibitor
Aatf	apoptosis antagonizing transcription factor
Zfp403	zinc finger protein 403
Ppm1d	protein phosphatase
Tbx2	T-box 2
Mpo	myeloperoxidase
Vezf1	vascular endothelial zinc finger 1
Akap1	A kinase (PRKA) anchor protein 1

Conclusions: Allele-specific genotyping by quantitative PCR can be used to identify QTL in pools of DNA from variably responsive F2 mice. DNA sequence comparison (5), together with microarray-based expression analysis (data not shown), can then reduce the number of candidate genes within these QTL. In our experiments, we studied PMN responses (data shown) as well as TNF-alpha responses (data not shown). This general approach should be applicable to studying any trait for which quantitative differences are observed between two inbred strains of mice.